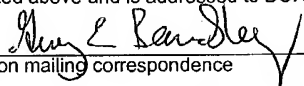
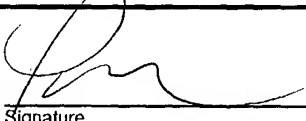



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Substitute Form PTO 1390 U.S. Department of Commerce Patent and Trademark Office TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		Attorney's Docket Number: <u>06727/010002</u> U.S. Application Number: <u>PCT/US00/24129</u>
INTERNATIONAL APPLICATION NUMBER	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/US00/24129	September 1, 2000	September 2, 1999
TITLE OF INVENTION:	USE OF rAFP TO INHIBIT OR PREVENT APOPTOSIS	
APPLICANTS FOR DO/EO/US:	Robert A. Murgita, Robert Mulroy, and Stace Lindsay	
Applicants herewith submit to the United States Designated/Elected Office (DO/EO/US) the following items and other information:		
1.	<input checked="" type="checkbox"/> This is a FIRST submission of items concerning a filing under 35 U.S.C. § 371.	
2.	<input type="checkbox"/> This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. § 371.	
3.	<input checked="" type="checkbox"/> This is an express request to begin national examination procedures (35 U.S.C. § 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. § 371(b) and PCT Articles 22 and 39(1).	
4.	<input checked="" type="checkbox"/> A proper Demand for International Preliminary Examination was made by the 19 th month from the earliest claimed priority date.	
5.	A copy of the International Application as filed (35 U.S.C. § 371(c)(2)). <input type="checkbox"/> a. is transmitted herewith (required only if not transmitted by the International Bureau). <input type="checkbox"/> b. has been transmitted by the International Bureau. <input type="checkbox"/> c. is not required, as the application was filed with the United States Receiving Office (RO/US).	
6.	<input type="checkbox"/> A translation of the International Application into English (35 U.S.C. § 371(c)(2)).	
7.	Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. § 371(c)(3)). <input type="checkbox"/> a. are transmitted herewith (required only if not transmitted by the International Bureau). <input type="checkbox"/> b. have been transmitted by the International Bureau. <input type="checkbox"/> c. have not been made; however, the time limit for making such amendments has NOT expired. <input checked="" type="checkbox"/> d. have not been made and will not be made.	
8.	<input type="checkbox"/> A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. § 371(c)(3)).	
9.	<input checked="" type="checkbox"/> An oath or declaration of the inventors (35 U.S.C. § 371(c)(4)).	
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11.	<input type="checkbox"/> An Information Disclosure Statement under 37 C.F.R. §§ 1.97 and 1.98.	
12.	<input type="checkbox"/> An assignment for recording. A separate cover sheet in compliance with 37 §§ 3.28 and 3.31 is included.	
13.	<input type="checkbox"/> A FIRST preliminary amendment. <input type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment.	
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17.	<p>■ The following fees are submitted:</p> <p>BASIC NATIONAL FEE (37 C.F.R. § 1.492(A)(1)-(5)):</p> <p>Neither international preliminary examination fee (37 C.F.R. § 1.482) nor international search fee (37 C.F.R. § 1.455(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO \$ 1040.00</p> <p>International preliminary examination fee (37 C.F.R. § 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO \$ 890.00</p> <p>International preliminary examination fee (37 C.F.R. § 1.482) not paid to USPTO but international search fee (37 C.F.R. § 1.445(a)(2)) paid to USPTO \$ 740.00</p> <p>X International preliminary examination fee (37 C.F.R. § 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1) - (4) \$ 710.00 \$ 710.00</p> <p>International preliminary examination fee paid to USPTO (37 C.F.R. § 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) \$ 100.00</p>		
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Surcharge of \$130 for furnishing the oath or declaration later than <input type="checkbox"/> 20 OR <input type="checkbox"/> 30 months from the earliest claimed priority date (37 C.F.R. § 1.492(e)).			\$
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE
Total claims	[**] - 20 =		x \$18 \$
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Multiple dependent claims (if applicable)			+ \$280 \$
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Robert A. Murgita et al.	Art Unit:	Not Yet Assigned
Serial No.:	<u>10/069,623</u>	Examiner:	Not Yet Assigned
Filed:	February 26, 2002	Customer No.:	21559
Title:	USE OF rAFP TO INHIBIT OR PREVENT APOPTOSIS		

Commissioner for Patents
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Prior to examination, please amend the application as follows.

In the Specification:

Please insert the following after the title.

--Cross Reference To Related Applications

This application claims priority under 35 U.S.C. § 371 to PCT Application

PCT/US00/24129, filed September 1, 2000, which claims priority from United States Provisional

Application No. 60/152,166, filed September 2, 1999.--

If there are any additional charges or any credits, please apply them to Deposit Account

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Respectfully submitted,

Date: January 29, 2003

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WO 01/15709

PCT/US00/24129

USE OF rAFP TO INHIBIT OR PREVENT APOPTOSIS

BACKGROUND OF THE INVENTION

5 The invention related to methods of inhibiting apoptosis.

There are two general ways in which cells die. The most easily recognized way is by necrosis, which is usually caused by an injury that is severe enough to disrupt cellular homeostasis. Typically, the cell's osmotic pressure is disturbed and, consequently, the cell swells and then ruptures.

10 When the cellular contents are spilled into the surrounding tissue space, an inflammatory response often ensues.

The second general way by which cells die is referred to as apoptosis, or programmed cell death. Apoptosis often occurs so rapidly that it is difficult to detect. This may help to explain why the involvement of
15 apoptosis in a wide spectrum of biological processes has only recently been recognized.

The apoptosis pathway has been highly conserved throughout evolution, and plays a critical role in embryonic development, viral pathogenesis, cancer, autoimmune disorders, and neurodegenerative disease.

20 For example, inappropriate apoptosis may cause or contribute to AIDS, Alzheimer's Disease, Parkinson's Disease, Amyotrophic Lateral Sclerosis (ALS), retinitis pigmentosa and other diseases of the retina, myelodysplastic syndrome (e.g. aplastic anemia), toxin-induced liver disease, including alcoholism, and ischemic injury (e.g. myocardial infarction, stroke, and
25 reperfusion injury). Conversely, the failure of an apoptosis response has been implicated in the development of cancer, particularly follicular lymphoma, p53-mediated carcinomas, and hormone-dependent tumors, in autoimmune

disorders, such as lupus erythematosus and multiple sclerosis, and in viral infections, including those associated with herpes virus, poxvirus, and adenovirus.

5 In patients infected with HIV-1, mature CD4⁺ T lymphocytes respond to stimulation from mitogens or super-antigens by undergoing apoptosis. However, the great majority of these cells are not infected with the virus. Thus, inappropriate antigen-induced apoptosis could be responsible for the destruction of this vital part of the immune system in early stages of HIV infection.

10

SUMMARY OF THE INVENTION

In general, the invention features the inhibition of apoptosis in a cell, e.g., a cell in a mammal such as a human patient, by contacting the cell with recombinant alpha-fetoprotein ("rHuAFP") or an effective fragment thereof, or
15 with nucleic acid encoding rHuAFP. The invention, in inhibiting apoptosis, can provide therapy for diseases in which inappropriate apoptosis is a feature, including AIDS or HIV infection, neurodegenerative diseases such as ALS, a myelodysplastic syndrome, or an ischemic injury such as occurs in stroke, myocardial infarction, reperfusion injury, or a toxin-induced liver disease.
20 Other features and advantages of the invention will be apparent from the detailed description of the invention, the drawings, and the claims.

BRIEF SUMMARY OF THE DRAWINGS

Fig. 1 is the nucleotide sequence (SEQ ID NO: 1) and deduced amino acid sequence (SEQ ID NO: 2) of the cDNA encoding human alpha-
25 fetoprotein, and the amino acid sequences (SEQ ID NOs: 3-8) of rHuAFP fragments.

Fig. 2 is the SDS-PAGE analysis of rHuAFP Fragment I (SEQ ID NO: 8) (Lane A, MW marker; Lane B, native human alpha-fetoprotein (AFP); Lane C, unpurified rAFP; Lane D, rAFP Fragment I, and Lane E, AFP (amino acids 1-590 of Fig. 1, SEQ ID NO: 2).

5

DETAILED DESCRIPTION OF THE INVENTION

Production of Recombinant Human Alpha-fetoprotein

Recombinant AFP can be produced in any standard recombinant protein production system, including prokaryotic cells such as *E. coli*, and eukaryotic systems such as yeast, mammalian (e.g., CHO cells) and insect cells.

10 Prokaryotic production of rHuAFP is described in Murgita U.S. Patent No. 5,384,250, hereby incorporated by reference.

The methods of the invention can also employ biologically active fragments of rHuAFP. A biologically active fragment of rHuAFP is one that possesses at least one of the following activities: (a) directs a specific
15 interaction with a target cell, e.g., binds to a cell expressing a receptor that is recognized by rHuAFP (e.g., the membrane of a cancer cell such as MCF-7); or (b) halts, reduces, or inhibits apoptosis (e.g., binds to a cell surface receptor and imparts an anti-apoptosis signal). The ability of rHuAFP fragments to bind to a receptor which is recognized by rHuAFP can be tested using any
20 standard binding assay known in the art.

In general, fragments of rHuAFP are produced according to the techniques of polypeptide expression and purification described in U.S. Patent No. 5,384,250. DNA sequences encoding fragments of rHuAFP can be generated by standard techniques and cloned into expression vectors for
25 expression in recombinant cells. Expressed fragments can be isolated by various chromatographic and/or immunological methods known in the art.

Lysis and fractionation of rHuAFP-containing cells prior to affinity chromatography may be performed by standard methods. Once isolated, the recombinant protein can, if desired, be further purified, e.g., by high performance liquid chromatography (see, e.g., Fisher, Laboratory Techniques
5 In Biochemistry and Molecular Biology, Work and Burdon, eds., Elsevier, 1980).

Recombinant HuAFP fragments can be assayed by immunological procedures, such as Western blot, immunoprecipitation analysis of recombinant cell extracts, or immunofluorescence (using, e.g., the methods
10 descrbed in Ausubel et al., *Current Protocols In Molecular Biology*, Greene Publishing Associates and Wiley Interscience (John Wiley & Sons), New York, 1994).

Useful rHuAFP fragments preferably have at least 20 contiguous amino acids, preferably at least 50 contiguous amino acids, more preferably at
15 least 100 contiguous amino acids, and most preferably at least 200 to 400 or more contiguous amino acids in length.

Recombinant HuAFP fragments of interest include, but are not limited to, Domain I (amino acids 1 (Thr) - 197 (Ser), see Fig. 1, SEQ ID NO: 3), Domain II (amino acids 198(Ser) - 389 (Ser), see Fig. 1, SEQ ID NO: 4),
20 Domain III (amino acids 390 (Gln) - 590 (Val), see Fig. 1, SEQ ID NO: 5), Domain I+II (amino acids 1 (Thr) - 389 (Ser), see Fig. 1, SEQ ID NO: 6), Domain II+III (amino acids 198 (Ser) - 590 (Val), see Fig. 1, SEQ ID NO: 7), and rHuAFP Fragment I (amino acids 266 (Met) - 590 (Val), see Fig. 1, SEQ ID NO: 8).

25 By "inhibiting apoptosis" is meant a decrease in the number of cells which undergo apoptosis relative to an untreated control. Preferably, the decrease is at least 25%, more preferably the decrease is 50%, and most

preferably the decrease is at least one-fold.

Apoptosis Assays

- Apoptosis assays are described in the following references. Assays for apoptosis in lymphocytes are disclosed by, for example: Li et al.,
- 5 "Induction of apoptosis in uninfected lymphocytes by HIV-1 Tat protein", Science 268:429-431, 1995; Gibellini et al., "Tat-expressing Jurkat cells show an increased resistance to different apoptosis stimuli, including acute human immunodeficiency virus-type 1 (HIV-1) infection", Br. J. Haematol. 89:24-33, 1995; Martin et al., "HIV-1 infection of human CD4⁺ T cells *in vitro*.
- 10 Differential induction of apoptosis in these cells." J. Immunol. 152:330-42, 1994; Terai et al., "Apoptosis as a mechanism of cell death in cultured T lymphoblasts acutely infected with HIV-1", J. Clin Invest. 87:1710-5, 1991; Dhein et al., "Autocrine T-cell suicide mediated by APO-1/(Fas/CD95) 11, Nature 373:438-441, 1995; Katsikis et al., "Fas antigen stimulation induces
- 15 marked apoptosis of T lymphocytes in human immunodeficiency virus-infected individuals", J. Exp. Med. 181:2029-2036, 1995; Estendorp et al., "Sensitization of T cells to CD95-mediated apoptosis by HIV-1 Tat and gp120", Nature 375:497, 1995; DeRossi et al., Virology 198:234-44, 1994.

- Assays for apoptosis in fibroblasts are disclosed by, for example:
- 20 Vossbeck et al., "Direct transforming activity of TGF-beta on rat fibroblasts," Int. J. Cancer 61:92-97, 1995; Goruppi et al., "Dissection of c-myc domains involved in S phase induction of NIH3T3 fibroblasts," Oncogene 9:1537-44, 1994; Fernandez et al., "Differential sensitivity of normal and Ha-ras transformed C3H mouse embryo fibroblasts to tumor necrosis factor: induction
- 25 of bcl-2, c-myc, and manganese superoxide dismutase in resistant cells," Oncogene 9:2009-17, 1994; Harrington et al., "c-Myc-induced apoptosis in

fibroblasts is inhibited by specific cytokines," EMBO J., 13:3286-3295, 1994;
Itoh et al., "A novel protein domain required for apoptosis. Mutational
analysis of human Fas antigen," J. Biol. Chem. 268:10932-7, 1993.

Assays for apoptosis in neuronal cells are disclosed by, for example:

- 5 Melino et al., "Tissue transglutaminase and apoptosis: sense and antisense
transfection studies with human neuroblastoma cells," Mol. Cell Biol.
14:6584-6596, 1994; Rosenbaum et al., "evidence for hypoxia-induced,
programmed cell death of cultured neurons," Ann. Neurol. 36:864-870, 1994;
Sato et al., "Neuronal differentiation of PC12 cells as a result of prevention of
10 cell death by bcl-2," J. Neurobiol. 25:1227-1234, 1994; Ferrari et al., "N-
acetylcysteine D- and L-stereoisomers prevents apoptosis death of neuronal
cells," J. Neurosci. 15:2857-2866, 1995; Talley et al., "Tumor necrosis
factor alpha-induced apoptosis in human neuronal cells: protection by the
antioxidant N-acetylcysteine and the genes bcl-2 and crmA," Mol. Cell Biol.
15 1585:2359-2366, 1995; Talley et al., "Tumor Necrosis Factor Alpha-Induced
Apoptosis in Human Neuronal Cells: Protection by the Antioxidant
N-Acetylcysteine and the Genes bcl-2 and crmA," Mol. Cell. Biol. 15:2359-
2366, 1995; and Walkinshaw et al., "Induction of apoptosis in
catecholaminergic PC12 cells by L-DOPA. Implication for the treatment of
20 Parkinson's disease," J. Clin. Invest. 95:2458-2464, 1995.

- Assays for apoptosis in insect cells are disclosed by, for example:
- Clem et al., "Prevention of apoptosis by a baculovirus gene during infection of
insect cells," Science 254:1388-90, 1991; Crook et al., "An apoptosis-
inhibiting baculovirus gene with a zinc finger-like motif," J. Virol. 67:2168-
25 74, 1993; Rabizadeh et al., "Expression of the baculovirus p35 gene inhibits
mammalian neural cell death," J. Neurochem. 61:2318-21, 1993; Birnbaum et
al., "an apoptosis inhibiting gene from a nuclear polyhedrosis virus encoding a

polypeptide with Cys/His sequence motifs," J. Virol. 68:2521-8, 1994; and Clem et al., "Control of programmed cell death by the baculovirus genes p35 and IAP," Mol. Cell. Biol. 14:5212-5222, 1994.

Gene Therapy

- 5 rHuAFP-encoding genes can be used according to the invention in anti-apoptosis gene therapy. In particular, a functional rHuAFP gene may be used to sustain neuronal cells that undergo apoptosis in the course of a neurodegenerative disease; lymphocytes (i.e., T cells and B cells); or cells that have been injured by ischemia.
- 10 Retroviral vectors, adenoviral vectors, adeno-associated viral vectors, or other viral vectors with the appropriate tropism for cells likely to be involved in apoptosis (for example, epithelial cells) may be used as a gene transfer delivery system for a therapeutic rHuAFP gene construct. Numerous vectors useful for this purpose are known (Miller, Human Gene Therapy 15-
15 15, 1990; Friedman, Science 244:1275-1281, 1989; Eglitis and Anderson, BioTechniques 6:608-614, 1988; Tolstoshev and Anderson, Current Opinion in Biotechnology 1:55-61, 1990; Sharp, The Lancet 337:1277-1278, 1991; Cornetta et al., Nucleic Acid Research and Molecular Biology 36:311-322, 1987; Anderson, Science 226:401-409, 1984; Moen, Blood Cells 17:407-416,
20 1991; Miller et al., Biotechniques 7:980-990, 1989; Le Gal La Salle et al., Science 259:988-990, 1993; and Johnson, Chest 107:77S-83S, 1995). Retroviral vectors are particularly well developed and have been used in clinical settings (Rosenberg et al., N. Engl. J. Med 323:370, 1990; Anderson et al., U.S. Patent NO. 5,399,346). Non-viral approaches may also be employed
25 for the introduction of therapeutic DNA into cells otherwise predicted to undergo apoptosis. For example rHuAFP may be introduced into a neuron or a

T cell by lipofection (Felgner et al., Proc. Natl. Acad. Sci. USA 84:7413, 1987; Ono et al., Neurosci. Lett. 117:259, 1990; Brigham et al., Am. J. Med. Sci. 298:278, 1989; Staubinger et al., Meth. Enz. 101:512, 1983),
asialorosonucoid-polylysine conjugation (Wu et al., J. Biol. Chem. 263:14621,
5 1988; Wu et al., J. Biol. Chem. 264:16985, 1989); or, less preferably,
microinjection under surgical conditions (Wolff et al., Science 247:1465,
1990).

For any of the methods described above, the therapeutic rHuAFP
DNA construct is preferably applied to the site of the predicted apoptosis event
10 (for example, by injection), or to tissue in the vicinity of the predicted
apoptosis event, or to a blood vessel supplying the cells predicted to undergo
apoptosis.

rHuAFP expression can be directed from any suitable promoter (e.g.,
the human cytomegalovirus (CMV), simian virus 40 (SV40), or
15 metallothionein promoters), and regulated by any appropriate mammalian
regulatory element. For example, if desired, enhancers that preferentially
direct gene expression in neural cells, T cells, or B cells may be used to direct
rHuAFP expression. Alternatively, if an rHuAFP genomic clone is used in a
therapeutic construct, regulation may be mediated by the cognate regulatory
20 sequences or, if desired, by regulatory sequences derived from a heterologous
source, including any of the promoters or regulatory elements described above.

Alternatively, rHuAFP gene therapy is accomplished by
direct administration of the rHuAFP mRNA or antisense rHuAFP mRNA to a
cell that is expected to undergo apoptosis. The mRNA may be produced and
25 isolated by any standard technique, but is most readily produced by *in vitro*
transcription using an rHuAFP cDNA under the control of a high efficiency
promoter (e.g., the T7 promoter). Administration of rHuAFP mRNA to cells

can be carried out by any of the methods for direct nucleic acid administration described below.

Ideally, the production of rAFP protein by any gene therapy approach will result in cellular levels of rAFP that are at least equivalent to the normal, cellular level of rHuAFP in an unaffected cell. Treatment by any rHuAFP-mediated gene therapy approach may be combined with more traditional therapies.

Administration of rAFP Polypeptides

Another therapeutic approach of the invention involves administration of recombinant rHuAFP, either directly to the site of a predicted apoptosis event (for example, by injection) or systemically (for example, by any conventional recombinant protein administration technique). The dosage of rHuAFP depends on a number of factors, including the size and health of the individual patient, but, generally, between 0.1 mg and 100 mg are administered per day to an adult in a pharmaceutically-acceptable formulation. Administration may begin before or after the patient is symptomatic. Any appropriate route of administration may be employed, for example, administration may be parenteral, intravenous, intraarterial, subcutaneous, intramuscular, intracranial, intraorbital, ophthalmic, intraventricular, intracapsular, intraspinal, intracisternal, intraperitoneal, intranasal, aerosol, or oral. Therapeutic formulations may be in the form of liquid solutions or suspensions; for oral administration, formulations may be in the form of tablets or capsules; and for intranasal formulations, in the form of powders, nasals drops, or aerosols.

Methods well known in the art of making formulations are found, for example, in *Remington's Pharmaceutical Sciences*, (18th edition), ed. A.

Gennaro, 1990, Mack Publishing Company, Easton, PA. Formulations for parenteral administration may, for example, contain excipients, sterile water, or saline, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, or hydrogenated naphthalenes. Biocompatible, biodegradable lactide polymer, lactide/glycolide copolymer, or polyoxyethylene-polyoxypropylene copolymers may be used to control the release of rHuAFP.

Treatment with an rHuAFP protein or gene may be combined with more traditional therapies for the disease such as surgery, steroid therapy, or chemotherapy for autoimmune disease; antiviral therapy for AIDS; and tissue plasminogen activator (TPA) for ischemic injury.

Other Embodiments

All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each independent publication or patent application was specifically and individually indicated to be incorporated by reference.

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure come within known or customary practice within the art to which the invention pertains and may be applied to the essential features hereinbefore set forth, and follows in the scope of the appended claims.

What is claimed is:

1. A method of inhibiting apoptosis in a cell, said method comprising administering to said cell an apoptosis inhibiting amount of rHuAFP or an apoptosis-inhibiting fragment thereof.
2. The method of claim 1, wherein said cell is in a mammal.
- 5 3. The method of claim 2, wherein said mammal is human.
4. The method of claim 3, wherein said human is infected with HIV, or has a neurodegenerative disease, a myelodysplastic syndrome, or an ischemic injury.
5. The method of claim 4, wherein said ischemic injury is
10 caused by a myocardial infarction, a stroke, a reperfusion injury, or a toxin-induced liver disease.
6. A method of inhibiting apoptosis in a cell, said method comprising transfecting said cell with nucleic acid encoding rHuAFP or an apoptosis-inhibiting fragment thereof.
- 15 7. The method of claim 6, wherein said cell is in a human patient.
8. The method of claim 7, wherein said human patient is infected with HIV, or has a neurodegenerative disease, a myelodysplastic syndrome, or an ischemic injury.

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- (71) Applicant (*for all designated States except US*): AT-
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ning of each regular issue of the PCT Gazette.*

(54) Title: USE OF rAFP TO INHIBIT OR PREVENT APOPTOSIS

(57) Abstract: A method of inhibiting apoptosis in a cell by administering to the cell an apoptosis inhibiting amount of recombinant human alpha-feta protein or an apoptosis-inhibiting fragment thereof.

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Fig. 1

211 phe gly thr arg thr phe gln ala ile thr val thr lys leu ser gln lys phe thr lys val asn phe thr glu ile gln lys leu val (821)
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 280
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 GAT GAA AAA CCT GAA GGT CTA TCT CCA AAT CTA AAC AGG TTT TTA GGA GAT AGA GAT TTT AAC CAA TTT TCT TCA GGG GAA AAA AAT ATC(1091)
 310
 331 phe leu ala ser phe val his glu tyr ser arg arg his pro gln leu ala val ser val ile leu arg val ala lys gly tyr gln glu
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 340
 361 leu glu lys cys phe gln thr glu thr glu asn pro leu glu cys gln asp lys gly glu glu leu gln lys tyr ile gln glu ser gln
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 370
 391 ala leu ala lys arg ser cys gly leu phe gln lys leu gly glu tyr thr leu gln asn ala phe leu val ala tyr thr lys lys ala
 GCA TTG GCA AAG CGA AGC TGC TGC CTC TTC CAG AAA CTA GGA GAA TAT TAC TTA CAA AAT GCG TTT CTC GTT GCT TAC ACA AAG AAA GCC(1361)
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 421 pro gln leu thr ser ser glu leu met ala ile thr arg lys met ala ala thr ala thr cys cys gln leu ser glu asp lys leu
 CCC CAG CTG ACC TCG GAG CTG ATG GCC ATC ACC AGA AAA ATG GCA GCC ACA GCA GCC ACT TGT TGC CAA CTC AGT GAG GAC AAA CTA(1451)
 430
 450

Fig. 1 (CONTINUED)

10/069623

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cys cys thr ser ser tyr ala asn arg arg pro cys phe ser ser leu val val asp glu thr tyr val pro pro ala phe ser asp asp			
TGC TGC ACT TCT TCA TAT GCC AAC AGG AGG CCA TGC TTC AGC AGC TTT GTC GAT GAA ACA TAT GTC CCT CCT GAT GAC(1631)			
511	520	530	540
lys phe ile phe his lys asp leu cys gln ala gln gly val ala leu gln thr met lys gln glu phe leu ile asn leu val lys gln			
AAG TTC ATT TTC CAT AAG GAT CTG TGC CAA GGT CAG GGT GTA GCG CTG CAA ACG ATG AAG CAA GAG TTT CTC ATT AAC CTT GTG AAG CAA(1721)			
541	550	560	570
lys pro gln ile thr glu glu gln leu glu ala val ile ala asp phe ser gly leu leu glu lys cys cys gln gly gln glu gln glu			
AAG CCA CAA ATA ACA GAG GAA CAA CTT GAG GCT GTC ATT GCA GAT TTC TCA GGC CTG TTG GAG AAA TGC TGC CAA GGC CAG GAA CAG GAA(1811)			
571	580	590	
val cys phe ala glu glu gly gln lys leu ile ser lys thr arg ala ala leu gly val ter			
GTC TGC TTT GCT GAA GAG GGA CAA AAA CTG ATT TCA AAA ACT CGT GCT GCT TTG GGA GTT TAA ATTACTTCAGGGGAAGAGACAAAACGAGTCT(1908)			
TTCAATCGGTGTGAACCTTTCTCTTTAATTTTAACCTTTTAACTGATTTTAACACTTTTGTGAAATTAATGAATGATAAGACCTTTTATGTGAGATTTCCCTTATCACAGAAATAAATAATCTCCAAA(2027)			

Fig. 1 (CONTINUED)

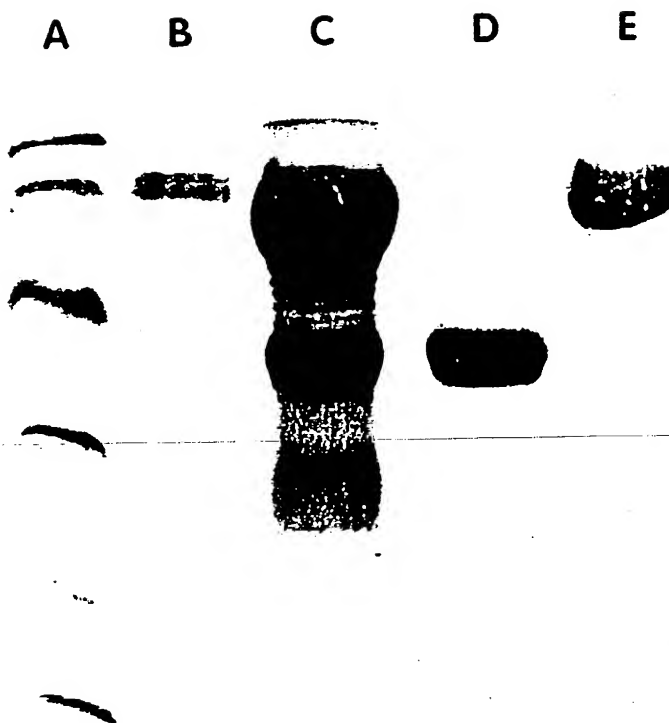


Fig. 2

PATENT

ATTORNEY DOCKET NO: 06727/010002

COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled USE OF RAFP TO INHIBIT OR PREVENT APOPTOSIS, the specification of which

~ is attached hereto.

: was filed on February 26, 2002 as Application Serial No. 10/069,623
and was amended on _____.

~ was described and claimed in PCT International Application No.
filed on _____ and as amended under PCT Article 19 on _____.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose all information I know to be material to patentability in accordance with Title 37, Code of Federal Regulations, § 1.56.

FOREIGN PRIORITY RIGHTS: I hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

Country	Serial Number	Filing Date	Priority Claimed?
PCT	PCT/US00/24129	September 1, 1999	Yes/No

PROVISIONAL PRIORITY RIGHTS: I hereby claim priority benefits under Title 35, United States Code, § 119(e) and § 120 of any United States provisional patent application(s) listed below filed by an inventor or inventors on the same subject matter as the present application and having a filing date before that of the application(s) of which priority is claimed:

Serial Number	Filing Date	Status
60/152,166	September 2, 1999	Pending



Dec-10-02 17:09

From-CLARK & ELBING LLP

617-237-1558

T-067 P.003/004 F-970

NON-PROVISIONAL PRIORITY RIGHTS: I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose all information I know to be material to patentability as defined in Title 37, Code of Federal Regulations § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

Serial Number	Filing Date	Status

I hereby appoint the following attorney and/or agents to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith: Paul T. Clark, Reg. No. 30,162, Karen L. Elbing, Ph.D. Reg. No. 35,238, Kristina Bleker-Brady, Ph.D. Reg. No. 39,109, Susan M. Michaud, Ph.D. Reg. No. 42,885, James D. DeCamp, Ph.D., Reg. No. 43,580, Sean J. Edman, Reg. No. 42,505, Vicki Healy, Reg. No. 48,343.

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Address all correspondence to: Paul T. Clark at Clark & Elbing LLP, 176 Federal Street, Boston, MA 02110. Customer No: 21559

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

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Nov-12-02 12:41

From-CLARK & ELBING LLP

617-237-1888

T-575 P.004/004 F-382

COMBINED DECLARATION AND POWER OF ATTORNEY

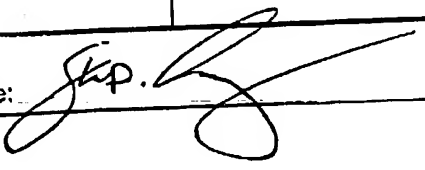
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Full Name (First, Middle, Last)	Residence Address (City, State, Country)	Post Office Address (Street, City, State, Country)	Citizenship
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Signature:			Date:

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Signature: 			Date: 11/21/02

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Murgita, Robert A.
Mulroy, Robert
Lindsay, Stace

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 Glu Ala Leu Ile Ala Asp Phe Ser Gly Leu Leu Glu Lys Cys Cys Gln
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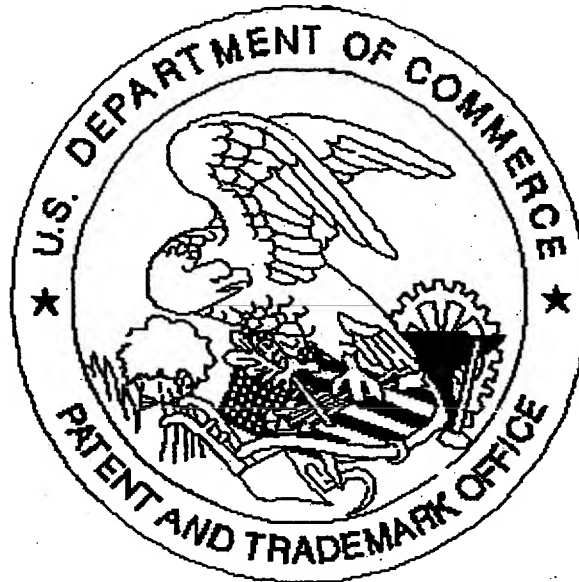
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 Ala Glu Asn Asp Glu Lys Pro Glu Gly Leu Ser Pro Asn Leu Asn Arg
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 Phe Leu Gly Asp Arg Asp Phe Asn Gln Phe Ser Ser Gly Glu Lys Asn
 50 55 60
 Ile Phe Leu Ala Ser Phe Val His Glu Tyr Ser Arg Arg His Pro Gln
 65 70 75 80
 Leu Ala Val Ser Val Ile Leu Arg Val Ala Lys Gly Tyr Gln Glu Leu
 85 90 95

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Leu	Glu	Lys	Cys	Phe	Gln	Thr	Glu	Asn	Pro	Leu	Glu	Cys	Gln	Asp	Lys
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Gly	Glu	Glu	Glu	Leu	Gln	Lys	Tyr	Ile	Gln	Glu	Ser	Gln	Ala	Leu	Ala
		115					120					125			
Lys	Arg	Ser	Cys	Gly	Leu	Phe	Gln	Lys	Leu	Gly	Glu	Tyr	Tyr	Leu	Gln
	130					135					140				
Asn	Glu	Phe	Leu	Val	Ala	Tyr	Thr	Lys	Lys	Ala	Pro	Gln	Leu	Thr	Ser
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Ser	Glu	Leu	Met	Ala	Ile	Thr	Arg	Lys	Met	Ala	Ala	Thr	Ala	Ala	Thr
			165						170					175	
Cys	Cys	Gln	Leu	Ser	Glu	Asp	Lys	Leu	Leu	Ala	Cys	Gly	Glu	Gly	Ala
		180						185					190		
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Ala	Phe	Ser	Asp	Asp	Lys	Phe	Ile	Phe	His	Lys	Asp	Leu	Cys	Gln	Ala
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Gln	Gly	Val	Ala	Leu	Gln	Arg	Met	Lys	Gln	Glu	Phe	Leu	Ile	Asn	Leu
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Val	Lys	Gln	Lys	Pro	Gln	Ile	Thr	Glu	Glu	Gln	Leu	Glu	Ala	Leu	Ile
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Ala	Ala	Leu	Gly	Val											
				325											

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☐ Page(s) _____ of _____ were not
present
for scanning. (Document title)

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black line.